EFFECTS OF BALLOON TAGS ON PERFORMANCE OF CHINOOK SALMON

Completion Report
for
U.S. Army Corps of Engineers MIPR: E96950042

March 15, 1996

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Executive Summary

1. Balloon-tagging significantly reduced the swimming performance of both the smaller fall-sized chinook (110-163 mm) and the larger spring-sized chinook (157-195 mm); however, the larger fish completed significantly more swim tests than did the smaller fish.

2. Elevated water temperature (16.4°C) reduced the overall swimming performance of the fall-sized chinook (control fish) but its effect on the balloon-tagged fish was not proportionately greater than for the cold water temperature (13°C).

3. The combined effects of anesthesia and handling (sham-tagging) had no significant effect on swim performance although the data suggest that the swimming ability of these fish is lessened slightly.

4. The procedures of anesthesia and handling, whether balloon- or sham-tagging, cause an immediate elevation of cortisol levels which drop significantly by 8 h (7.5 h after tag removal or last handling) after start of experiment . Fish do not totally recover by 24 h but cortisol levels are not much higher than normal physiological values.

5. Size of fish (fall- vs spring-sized) and water temperature (13 vs 16°C) were not significant factors in the primary stress response (cortisol).

6. The secondary stress response, as measured by glucose levels, is significally higher at 8 h for the fish that had worn the balloon tags than for the sham-tagged or control fish. By 24 h after start of the experiment, glucose levels of the balloon-tagged fish are equivalent to those of the sham-tagged fish within the same size or temperature group.

7. Glucose levels remain significantly elevated at 8 and 24 h (7.5 and 23.5 h, respectively, after last handling/tag removal) for both sham- and balloon-tagged fish from 16°C water in contrast to those of fish from 13°C. However, size of fish does not affect glucose.

8. Lactate, another measure of the secondary stress response, returned to pre-stress levels within 8 h (7.5 h after last handling/tag removal) for both sizes of the sham-tagged fish (13 and 16°C) and the balloon-tagged fish (13°C). However, the lactate levels of balloon-tagged fish acclimated to 16 C remained significantly elevated at 8 and 24 h.

9. After the balloon tags were removed, the fish recovered well physically, with minimal scarring and muscle damage.

10. Physiological stress test analyses (cortisol) revealed no differences between the Heisey RMC Environmental Services and the Oregon Cooperative Fisheries Research Unit (OCFRU) teams in using the balloon tag methodology.

Goals and Objectives

Balloon tags are employed to evaluate the effects of passage of juvenile salmonids through the Columbia and Snake River dams. The overall goal of this project was to evaluate the effect of this tagging methodology on the performance of spring and fall chinook. Specific objectives were to:

1. Determine how long it takes for the fish to recover from the stresses of the tagging procedure.
2. Determine the effects of the anesthetic alone employed in the tagging procedure in order to evaluate the consequences of cumulative effects of constituent components of the tagging procedure.
3. Determine how long it takes for the fish to recover from the stresses of the anesthetic alone.
4. Determine the effects of the tags on the swimming performance of fall and spring chinook.
5. Evaluate whether or not water temperature affects the performance of the fish relative to the tags.
6. Evaluate if there are run or fish size differences in response to the tags.

Methods

Fish. --Two fish sizes were used to compare the differences between the larger (spring) and smaller (fall) chinook at outmigration. Fish were reared and tested at the Fish Peformance and Genetics Laboratory at Oregon State University. The spring-sized chinook salmon were raised from eggs obtained from the Dexter Hatchery. These fish averaged 179 mm and 65 g at the time of the swimming performance tests. The fall-sized chinook salmon were raised from eggs obtained from the Marion Forks Hatchery and averaged 125 mm and 24.3 g at the time of the swim testing. For the experiments, all fish were kept in five foot circular tanks (70-90 fish/tank) and acclimated to their environment for at least two weeks. The fall-sized chinook were kept at both 13 and 16.4°C to simulate the possible temperature differences experienced by the fall chinook run during the summer months. The warm water fish were acclimated from 13°C to 16.4°C by increasing the water temperature 1°C per day. These fish remained at 16.4°C for at least two weeks before being subjected to testing.

Swim performance tests.--Each fish group (spring-sized fish, fall-sized fish at 13°C, and fall-sized fish at 16.4°C) were kept in replicate tanks. Fish were fasted 24 hours prior to testing. Care was taken to duplicate the timing and procedures used by Paul Heisey and his crew (RMC Environmental Services, Inc., Drumore, Pennsylvania) for tagging the fish with the Hi-Z Turb'N Tag under field conditions. To evaluate the effects of tagging and anesthesia, the spring- and fall-sized chinook (at both 13 and 16.4°C) were treated as negative controls (C-), positive controls (C+) or tagged (T+). At time 0, two fish were netted and either put into anesthesia (C+, T+) or into fresh water (C-) The C- fish were immediately placed into one of the modified Blaska respirometer-stamina chambers (Barton and Schreck, 1987; Smith and Newcomb, 1970) and were left undisturbed until the actual swim test. The C+ and T+ fish were anesthetized to a light sleep using 50 mg of buffered tricaine methanesulfonate (MS 222) per L, weighed and measured. The T+ fish were tagged with two balloon tags and a radiotag (approximately 5 g of weight) according to the procedures used by Heisey et al (1995). The C+ fish were not tagged but did receive handling (sham-tagging) equivalent to that of the T+ fish. Within 10 minutes of netting, the C+ and T+ fish were placed in the swim chambers where they were left undisturbed, with the water velocity at a speed that allowed the fish to orient themselves (0 to 0.4 bodylengths/sec). Just prior to the swim test, the swim chambers were disconnected from the motor and briefly inverted ($\leq$1 min). This handling simulated the second stressor, activation of balloon inflation, performed by Heisey's team before release. At 33 to 36 minutes after netting, the velocity in the swim chambers was increased to one bodylength per sec (BL/sec). When the fish were in swimming position, the velocity in the swim chamber was increased 1 BL/sec over 3 second intervals until the maximum swimming speed allowable in the chambers was reached (6 and 10 BL/sec, for the spring- and fall-sized chinook, respectively). The performance of the T+ fish with uninflated balloons was evaluated. The swim test ended when a fish flattened against the back baffle and could no longer struggle to regain swim position. For all fish, the duration and velocity of swim was recorded. For data analysis, a swim test was considered successfully completed if the fish swam at 6 BL/sec at 15 sec, a test which allowed comparison of the spring- and fall-sized chinook. In addition, because of their smaller size, the final BL/sec attained by the fall-sized chinook could also be analysed as a measure of swim performance. Usually, both swim chambers were operating in tandem so that combinations of the C- and T+, C+ and C-, and T+ and C+ treatments could be alternated throughout the day. An effort was made to run equal numbers of all treatments from each replicate tank in each swim chamber during the day. After the swim, the tags were removed from the T+ fish and all

tested fish were put into two foot tanks where they were maintained for two to five weeks before necropsy examination.

Physiological Stress Tests.--The same fish groups and treatments, as defined above, were used to evaluate the physiological stresses of anesthesia and sham- or balloon-tagging. Every effort was made to duplicate the timing and handling expected under actual field conditions. A slight temperature decrease (from 16.4 to 16°C within 24 h of testing) for the warm water fall-sized chinook was necessary to maintain adequate water flow for the extra tanks used but otherwise all conditions were similar to those used for the swimming performance test.

After acclimation for at least two weeks at the appropriate temperatures, four to six fish from each fish group were sampled for blood beginning at 0 h and continuing at 35 min, 8 and 24 h thereafter. Each fish group was run in triplicate, from three tanks, and yielded sample sizes up to 18 fish per treatment per time period. Care was taken to quietly and quickly net and anesthetize the fish (50 mg/L buffered MS 222) within 1 min to minimize stress from netting. The first four to six fish netted from a tank were immediately euthanized (200 mg/L buffered MS 222) and represented the unhandled C- fish at time 0. The next fish netted (C+) were anesthetized to a light sleep stage and sham-tagged. Four to six C+ fish were euthanized and bled to ascertain the effects of handling and tagging at 0 h (approximately 5-7 min after netting). The T+ fish were not sampled at the 0 h on the premise that physiological changes would be indistinguishable from those of the C+ fish at this time. The remaining fish from the tank were anesthetized, handled, and alternately sham-tagged (C+) or balloon-tagged (T+) in groups of 4-6 animals. Within 10 min of netting all fish had been treated and apportioned, by 2-3 animals, into two foot tanks with 50 L of slowly circulating water. At 33-37 min (0.5 h), the C+ and T+ fish were handled to simulate the procedure of balloon inflation. At this time, 4-6 fish from each treatment were euthanized for sampling at 0.5 h. The remaining fish were then lightly anesthetised within the tank (125 ml MS 222/tank for 1 min) and then either handled to remove the balloon tags (T+) or to simulate tag removal (C+) at 0.5 h. These fish were returned to their home or similar tank, with the C+ and T+ in separate tanks, for euthanasia and sampling at 8 or 24 h. After the first sampling at 0 h, subsequent samplings of negative control fish (C-) for determination of basal physiological levels at 8 and 24 h were taken from an undisturbed tank containing fish at the appropriate temperature and size.

At each time period, plasma was collected from the caudal vasculature using 250 µl heparinized capillary tubes and the fish were necropsied for tag effects and general pathology. Plasma cortisol was measured using a radioimmunoassay (Redding et al.

1984), while plasma glucose and lactate were measured by colorimetric procedures (Barton et al. 1986; Passonneau 1974; Wedemeyer and Yasutake 1977) to evaluate the primary and secondary stress responses of the fish.

A preliminary test, similar to the one above, was performed to validate our group's (Oregon Cooperative Fishery Research Unit or OCFRU) ability to tag fish with the Hi-Z Turb 'N Tags compared with Heisey's team. Using fall-sized chinook (13°C), each team alternately netted and sham-tagged (C+) or balloon-tagged fish (T+) from replicate tanks. Fish were euthanized at 0 h or placed in 2 ft tanks (6 animals/tank) and euthanized at 45 min or at 24 h. The balloon tags were removed from half of the fish (T-) at 45 min and the other half remained tagged (T+) through 24 h. At each time point, six fish from each treatment were sampled for plasma to evaluate cortisol levels.

Statistics.--Comparisons were performed to find the differences between the three treatments (C-, C+, and T+) within each fish group. To ascertain the effects of size and temperature on treatment, the spring- and fall-sized fish at 13°C and the fall-sized fish at 13 and 16°C, respectively were compared (the fall-sized chinook, 16°C, were not compared to the spring-sized chinook, 13°C). Chi-square tests were employed to evaluate the effects of tag implantation on the swimming ability of the spring-sized chinook and to compare fall- and spring-sized chinook. General linear models (GLM) that included all possible interactions for the class variables and testing of the main class variables by Bonferroni T tests were used to further examine the effects of treatments on the swimming ability of the smaller fall-sized chinook which could be swum to exhaustion. A regression analysis was performed to ascertain the correlation between the forklength, weight, or condition and the treatment (C+, T+, C-).

General linear modeling was also used to examine the effects of tagging on physiological stress as measured by plasma cortisol, glucose, and lactate at the different timepoints. Bonferroni T tests identified significant differences between timepoints, treatments, and fish groups.

For all tests, nonsignificance is when $P>0.05$. Mean values are reported with standard error.

Results and Discussion

Swim Performance Tests

The larger spring-sized chinook tolerated the balloon tags better than the smaller fall-sized chinook; however, fewer of the balloon-tagged fish in this group successfully completed the swim test (Table 1, Figure 1; $P<0.05$) compared to the control fish. For the spring-sized chinook, there were no significant differences between the unanesthetized, unhandled C- fish and the anesthetized, sham-tagged C+ fish but slightly fewer of the C+ fish completed the swim. The swimming space in the swim chambers was limited for the larger fish and this factor combined with the stresses of handling, may have contributed to the reduced swimming success of the balloon-tagged fish.

The smaller fall-sized chinook did not tolerate the balloon tagging well (Figure 1) and completed significantly fewer swim tests than did the spring-sized tagged fish ($P<0.05$) and the fall-sized control fish ($P<0.0001$, Table 2). The balloon-tagged fish also swam at a significantly reduced velocity (3.9 ± 0.3 BL/sec) compared with the sham-tagged and the unhandled control fish (7.3 ± 0.4 and 7.6 ± 0.4 BL/sec, respectively). Elevated water temperature (16.4°C) did not affect the swimming performance of the tagged fish compared with the fall-sized fish at 13°C (Figure 2).

The procedure of anesthesia and sham-tagging did not significantly affect the swimming performance of the spring- or fall-sized fish although the C- fish always successfully completed more swim tests than did the C+ fish (Table 3). There were no differences between the spring- and fall-sized chinook that swam at 13°C. However, swimming success, as measured by BL/sec, was considerably lessened ($P<0.05$) for the fall-sized control fish at 16.4°C (Figure 2). The reduced swimming success of the C- and C+ fish in warm water appeared to be affected by the particular swim chamber. The motor for swim chamber 2 was louder and possibly created more vibration as the velocity was increased. This may have agitated these fish whose behavior appeared more skittish than that of the fish in the colder water. Other factors, as operator error and test times, were ruled out because the swim tests for the cold and warm water fish were alternated on a daily basis. From these results, it appears that if there were no other environmental stressors, fish acclimated to 16.4°C swam as well as those fish acclimated to 13°C, a physiologically more comfortable temperature (Barton and Schreck, 1987). However, the fish in the warmer water, unlike the fish in the colder water, seemed to be measurably bothered by minor stressors (such as increased vibration and/or noise) which resulted in

poorer swim performances. Oddly, the unhandled C- fish were affected as much as the sham-tagged fish.

After completion of the swim tests, all fish including the those that had been tagged recovered quickly. Most were quietly swimming within 15 min and none required more than 2 h to recover. No bacterial or fungal infections at the two tag insertion sites were evident during or after a two to five week observation period of the fish. Necropsy revealed no gross pathological changes internally. For most fish, there was minimal external scarring and no apparent deep tissue damage at the tag insertion sites.

Physiological Stress Tests

Primary Stress response. There were no differences between the Heisey RMC Environmental Services and the OCFRU teams in tagging the fish, as based on physiological stress test analyses of cortisol levels (Figure 3). Thus, the methodology of balloon tag attachment was successfully transferred to and duplicated by OCFRU which ensured fair testing of the ballon tagging procedure for this study. For this preliminary test, some fish wore balloon tags for 24 h, resulting in cortisol levels higher (167-200 ng/ml) than those seen at 45 min, indicative of a chronic stress. The radiotag antennas entangled fish and several died in the interim. This indicates the impracticality of long-term wearing of the balloon tags with the radiotag. Under actual field conditions to evaluate passage of juvenile salmonids, the balloon tag apparatus is removed from the fish no later than 1 h after application.

Follow-up experiments comparing spring- and fall-sized chinook at 13°C and fall-sized chinook at 13 and 16.4°C allowed a more thorough analysis of the effects of balloon tagging on the primary stress response as measured by plasma cortisol levels in the fish. At time 0, the effect of anesthesia and handling (weighing and sham-tagging) were assessed in comparison to the unhandled negative controls which were netted and immediately killed. The cortisol levels were elevated immediately for the sham-tagged fish, significantly ($P \leq 0.002$) for two of the three fish groups (Figure 4). This reflected the immediate response of the adrenocortical system after the application of handling, even with anesthesia. At 35 min, immediately after a second handling of the C+ and T+ fish, cortisol was significantly elevated ($P<0.0001$) above pre-stress levels (Table 4). However, there were no significant differences between sham-tagging and the more physically intrusive procedure of balloon-tagging and, with one exception (the fall-sized chinook, 13°C, at 8 h), the cortisol values obtained for the C+ and the T+ fish did not differ significantly at any timepoint nor for any fish group (Figure 4, Table 4). By 8 h (an

evening sampling between 5 and 10 pm), the cortisol levels of fish in both treatments dropped ($P<0.0001$) from the peak values at 35 min to normal or high-normal values. However, the cortisol levels for T+ fish, while only slightly higher than the C+ fish, were significantly higher than for the C- fish. At 24 hr, cortisol values for both the sham- and balloon-tagged fish were elevated (ranging from 36.1 ± 7.3 to 60.2 ± 7.3 ng/ml) but this was insignificant from the 8 h values for all but the balloon-tagged fish acclimated at 16°C. Cortisol values for the spring-sized C+ and for the T+ from all three fish groups did not return to pre-stress levels by 8 or even 24 h after treatment. At 24 h, the unhandled controls for the three fish groups had cortisol values ranging from 5.5 to 17 ng/ml, significantly lower than those of the sham- or balloon-tagged fish. The slightly elevated cortisol levels for the sham- and balloon-tagged, while just above physiological normalcy, seem to indicate that these fish are more sensitive to minor environmental stressors (as netting) than the fish which did not undergo anesthesia and handling.

Neither water temperature nor fish size appeared to affect cortisol values (Table 4). The spring-sized chinook weighed more than double that of the fall-sized chinook and swam easily with the attached balloon tags; nonetheless, cortisol levels were equivalent or within normal physiological parameters for both groups of fish at all timepoints. There were also no differences in cortisol values between fish adapted to different water temperatures (13 vs 16°C), an observation noted previously by Barton and Schreck (1987).

Secondary stress responses. A measure of the secondary stress response, levels of plasma glucose were slower to rise than cortisol but increased significantly by 35 min for both the sham- and balloon-tagged fish (Figure 5, Table 5). By 8 h, the glucose levels in the C+ fish, although elevated, were insignificantly different from those of the C- fish while those of the T+ fish had increased and were significantly higher ($P<0.05$). At 24 h, the glucose levels for for most animals, regardless of treatment, were higher than the pre-stress glucose levels. The values for the C+ and T+ spring-sized chinook, while significantly elevated above those of the C- at 24 h, were insignificantly different from those of the fall-sized chinook (13°C). The fall-sized fish at 13°C (C-, C+, and T+) had normal, albeit higher-than-pre-stress levels of glucose. However, the fish in 16°C water, whether subjected to sham- or balloon-tagging, had the highest glucose levels at 8 h which continued significantly elevated at 24 h compared with the fish in the 13°C water. This cumulative increase in glucose, as sustained through 24 h, may indicate increasing demands for energy in response to stress (Barton and Schreck, 1986; Barton and Schreck, 1987). In another study examining the influence of temperature on carbohydrate stress response in juvenile chinook salmon adapted to 21°C (Barton and Schreck, 1987), glucose

was significantly elevated at one through twelve hours after acute stress but returned to normal levels within 24 h. Like cortisol, glucose increases in response to physical disturbances.

The curves of the lactate values were similar to those of the cortisol. At 0 h, the additional stress of anesthesia and handling caused a significant increase ($P<0.05$) in the lactate values of the C+ fish above that of the unhandled C- fish (Figure 6, Table 6). Lactate values continued to rise for both the C+ and T+ treatments, and were significantly elevated above normal at 0.5 h ($P<0.05$). By 8 h, lactate levels of most fish had returned to and remained at levels equivalent to those of the unhandled control fish. This was a characteristic response to acute stress as previously noted by other researchers (Barton and Schreck, 1986; Barton and Schreck, 1987; Pickering et al., 1982; Waring et al., 1992). The exception were the balloon-tagged fish acclimated to 16°C. These fish sustained a significantly higher level of lactate at 8 and 24 h compared with fish at the lower temperature (Table 6). Size was not a factor in lactate differences.

General Discussion

It appears that the procedures of balloon-tagging followed by removal of tag or sham-tagging are stress events from which the fish do not entirely recover within 24 hours. While cortisol levels appear to normalize at 8 h, there is a increase at 24 h which may reflect a post-stress sensitivity to minor environmental disturbances. Of the two procedures, balloon-tagging appears to be slightly more stressful as judging from the elevated levels of glucose at 8 h. However, for fish acclimated to 13°C, there are no physiological indicators that differ for the balloon- and sham-tagged fish at 24 h which indicates that balloon-tagged fish recover quickly from the more physically intrusive procedure.

The size of the fish, fall- or spring-sized, was not a factor in physiological response but water temperature was. In fish acclimated to 16°C, the cortisol levels were not significantly different from those of the fish in 13°C; however, the secondary stress response, signified by sustained high levels of glucose and lactate at 24 h, indicated that these fish responded with more intensity and reflected the metabolic cost of physical stress. Physical disturbances, such as enforced exercise or handling, tends to induce elevations in plasma lactate and glucose levels in salmonids but these normally return to pre-stress levels within 3 to 24 h (Barton and Schreck, 1987; Pickering et al., 1982; Vijayan and Moon, 1992; Waring et al, 1992).

In examining the effect of balloon tags on swimming ability, a test that occurred when cortisol levels had potentially peaked, the size of the fish was a definitive factor whereas water temperature was not. Significantly more of the balloon-tagged spring-sized chinook completed the swimming test than did the smaller fall-sized chinook; the smaller fish, regardless of water temperature swam poorly with balloon tags. However, for both fish sizes, the additional drag and, perhaps, unfamiliarity of balloon tag apparatus significantly decreased either the ability and/or willingness of the fish to perform in burst speed swim tests.

The sham-tagged fish, despite being subjected to anesthesia and handling, swam capably and completed as many swim tests as did the unhandled controls. In addition, the smaller fall-sized fish completed as many swim tests as the larger spring-sized fish after sham-tagging. The results for fish acclimated to warm (16.4°C) water were mixed; while half of the sham-tagged and unhandled control fish swam as well as the control fish at 13°C, the other half did not. The poor swimming performance was directly attributed to the particular swim chamber used and suggests that cumulative stressing factors, as noise or vibration, along with a warmer water temperature reduce swimming ability. In fact, the additional stresses of anesthesia and handling were inconsequential in comparison. The anesthesia may have had a calming influence on these fish which appear more easily frightened into panicky swims than the fish in cooler water.

Conclusions

The burst speed swim tests clearly demonstrated that size was a factor in the ability of fish to swim while wearing the balloon tags. The large fish with balloon tags swam capably. In contrast, the smaller fish swam poorly while wearing the tags, especially when the velocity of the water was greater than 4 BL/sec. However, like the large fish, all small fish tagged with balloons were able to maneuver in low water velocity ($\leq$2 BL/sec), especially when not confined in the swim chamber. During actual passage tests, the ability of the fish to swim may be inconsequential if the fish do not have to maneuver into position. Most fish, after recovering from the tagging procedure, upright themselves and maintain this position under low water flow. Presumably, in passage tests through the turbines, the velocity of the water will be enough to render directed swimming unlikely and because the balloons should inflate immediately after, there is little opportunity for the fish to actively swim. If fish in actual test situations are released into circumstances

where they can affect their entry positions into turbine units, for example, then the tags may present more of a problem, particularly for the smaller fish.

Balloon-tagging under cool water temperatures for fall- and spring-sized chinook salmon juveniles does not promote a physiological stress response much greater than that of anesthesia and handling. Presumably, these fish could be released into the environment with little problem after 8 to 24 h because the fish quickly recover swimming skills after the balloon tags are removed. However, tagging of fish that have acclimated to warmer water temperatures ($\geq$16^{o}C) produces fish that sustain abnormally high secondary stress responses at 24 h. The fact that even the unstressed fish at 16^{o}C are quickly affected by minor stresses that do not disturb fish at 13^{o}C and swim more poorly predicts a poor swimming performance and lessened ability to recover physiological normalcy. Thus, the long-term survival of such fish when released into natural waters, after completion of balloon tag studies, is more suspect.

Despite the potentially debilitating effects of warm water on the fish, none of the fish died from the handling and balloon-tagging procedures which closely imitated the tagging operations in the field, excluding the actual passage tests. Two to five weeks after the burst speed swim tests, following recovery in flow-through tanks with active water turnover, the fish showed no signs of infection and little scarring at the balloon tag sites. This suggests that fish recover with minimal long-term side effects from the balloon-tagging.

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Table 1. Ability of spring-sized chinook smolts to complete the swim test (attainment of 6 BL/sec within 15 seconds) at 13°C. The average forklength was 179 mm. The controls include the C- fish (unanesthetized negative control) and the C+fish (anesthetized and sham-tagged). The T+ fish received anesthesia and balloon tags.

Treatment	Total no.	No. completing test	% Completion	Probability that treatments differ
All Controls[1]	38	30	78.9	$P \leq 0.04$
T+	19	10	52.6	

[1]There is no significant difference between the C- and the C+ fish so these were combined into one group.

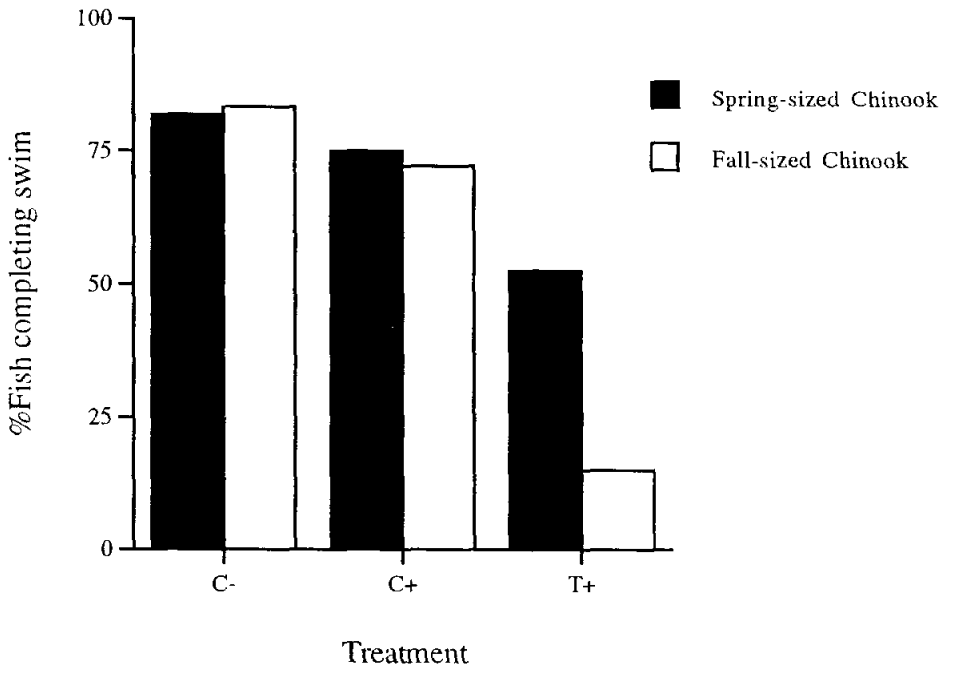

Figure 1. The percentage of spring- and fall-sized chinook that successfully completed swim tests at 6 BL/sec for a 15 second duration at 13 C. There was a significant difference ($P \leq 0.05$) between the balloon-tagged spring- and fall-sized chinook. Each treatment group consisted of 16 to 22 fish. C-=negative controls, C+=sham-tag controls, and T+=balloon-tagged fish.

Table 2. Ability of fall-sized chinook smolts to complete swim test (attainment of 6 BL/sec within 15 seconds) at 13°C. The average forklength was 125 mm. The C- fish are the unanesthetised negative control, the C+ fish received anesthesia and sham-tag handling, and the T+ fish received anesthesia and balloon tags.

Treatment	Total no.	No. completing test	% Completion	Probability that treatments differ
C-	18	15	83.3	
C+	18	13	72.2	$P<0.0001$
T+	20	3	15.0	

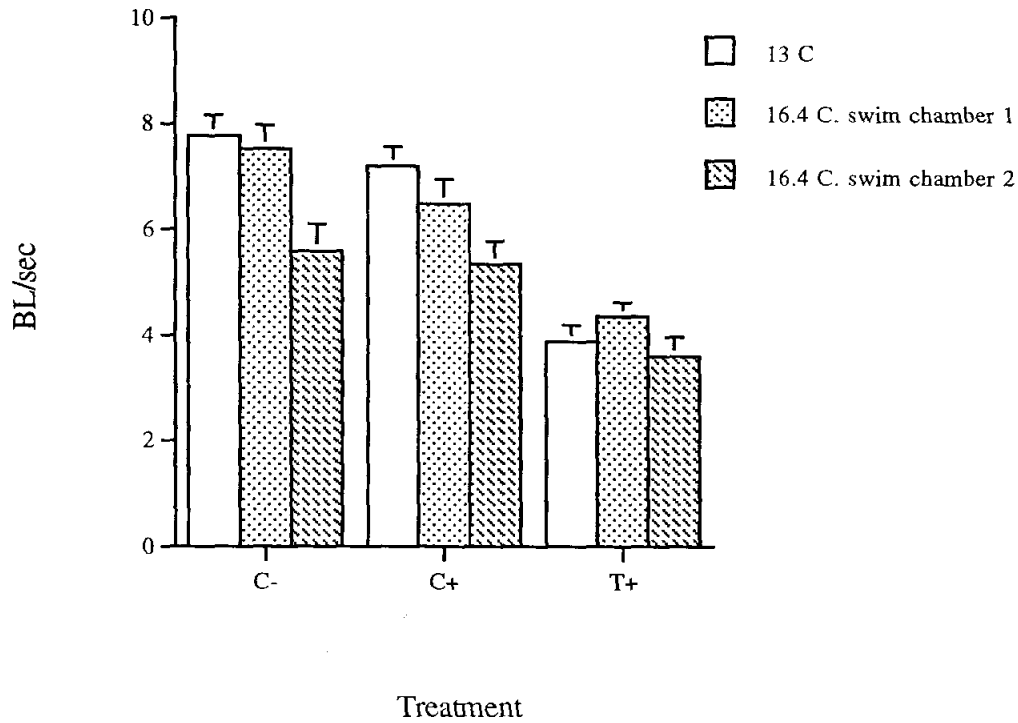

Figure 2. The average velocity (BL/sec) attained by fall-sized chinook smolts at 13 and 16.4 C. The swim chamber (1 and 2) had a significant effect ($P \leq 0.05$) on swim tests for the control fish in warm water but not for the control fish in cold water. The fish with balloon tags swam poorly at either temperature. Each treatment group consisted of 16 to 22 fish. C-=negative controls, C+=sham-tag controls, and T+=balloon-tagged fish.

Table 3. The effect of anesthesia and sham-tagging on the ability of spring- and fall-sized chinook smolts to complete swim tests (attainment of 6 BL/sec within 15 seconds) at 13 and 16.4 °C. The negative control fish, C-, received no anesthesia and minimal handling. The positive control fish, C+, received anesthesia and sham-tag handling. The average forklength was 179 mm for the spring-sized chinook and 125 mm for the fallsized chinook.

Fish size	Temperature (°C)	Treatment	Total no.	No. completing test	% Completion	Probability that treatment is significant[1]
Spring	13	C-	22	18	81.8	P>0.05
		C+	16	12	75.0	
Fall	13	C-	18	15	83.3	P>0.05
		C+	18	13	72.2	
Fall	16.4	C-	20	14	70.0	P>0.05
		C+	18	9	50.0	

[1]Comparisons for significance between C- and C+ treatments were made for each fish size/temperature group.

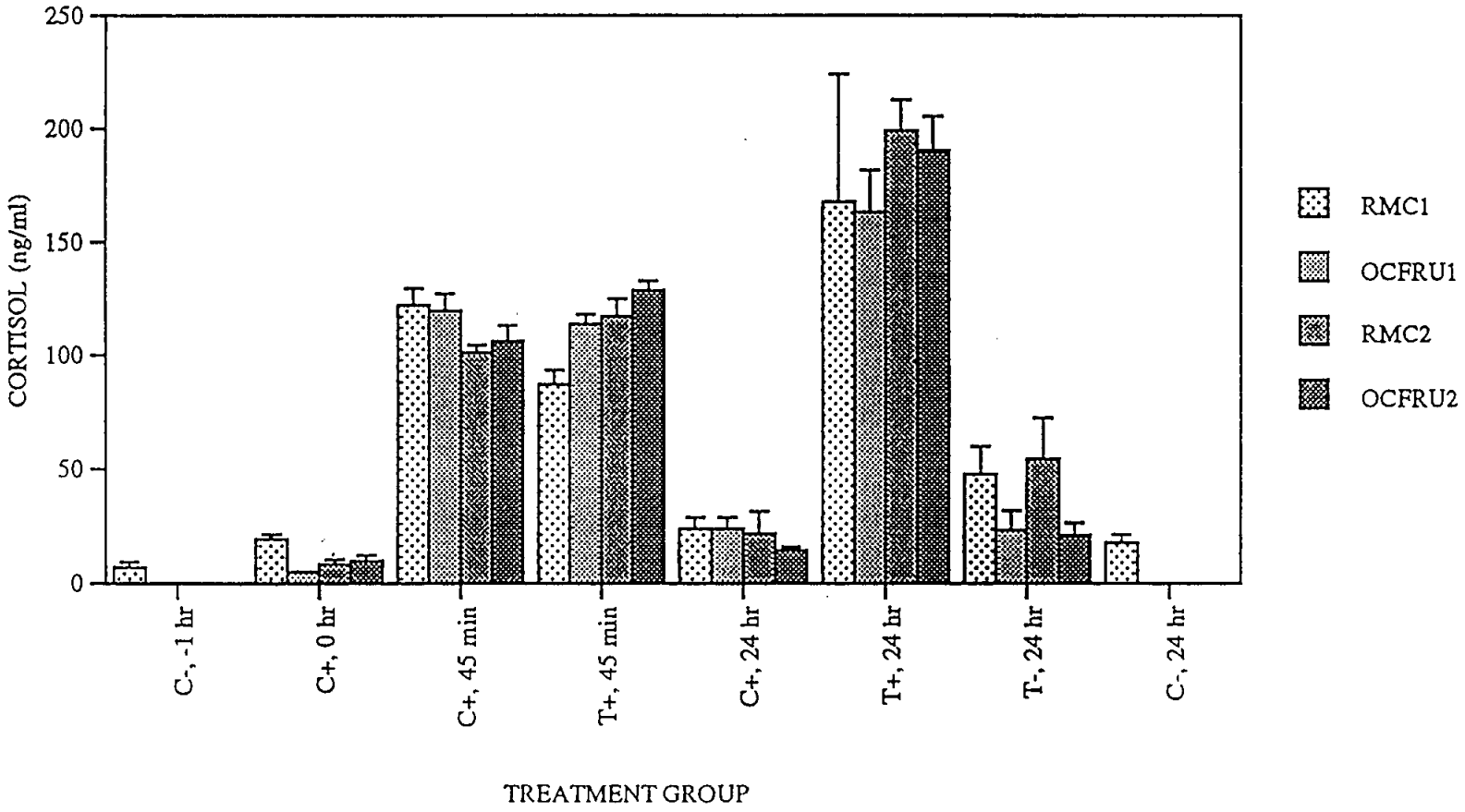

Figure 3. Comparison of the tagging abilities of the Heisey RMC Environmental Services (RMC1=tank 1, RMC2=tank 2) and the Oregon Cooperative Fishery Research Unit (OCFRU1=tank 1, OCFRU2=tank 2) teams as based on the physiological stress (measured by the cortisol levels) of fall-sized chinook smolts at 0 and 45 min, and 24 h. The C- fish are the unhandled, unanesthetized negative controls, and the C+ fish are the anesthetized and sham-tagged positive controls. The T+ and T- fish were balloon-tagged at 0 min. At 45 min, these fish (denoted as T+) were either detagged (T-) or left tagged (T+) until sampling at 24 h.

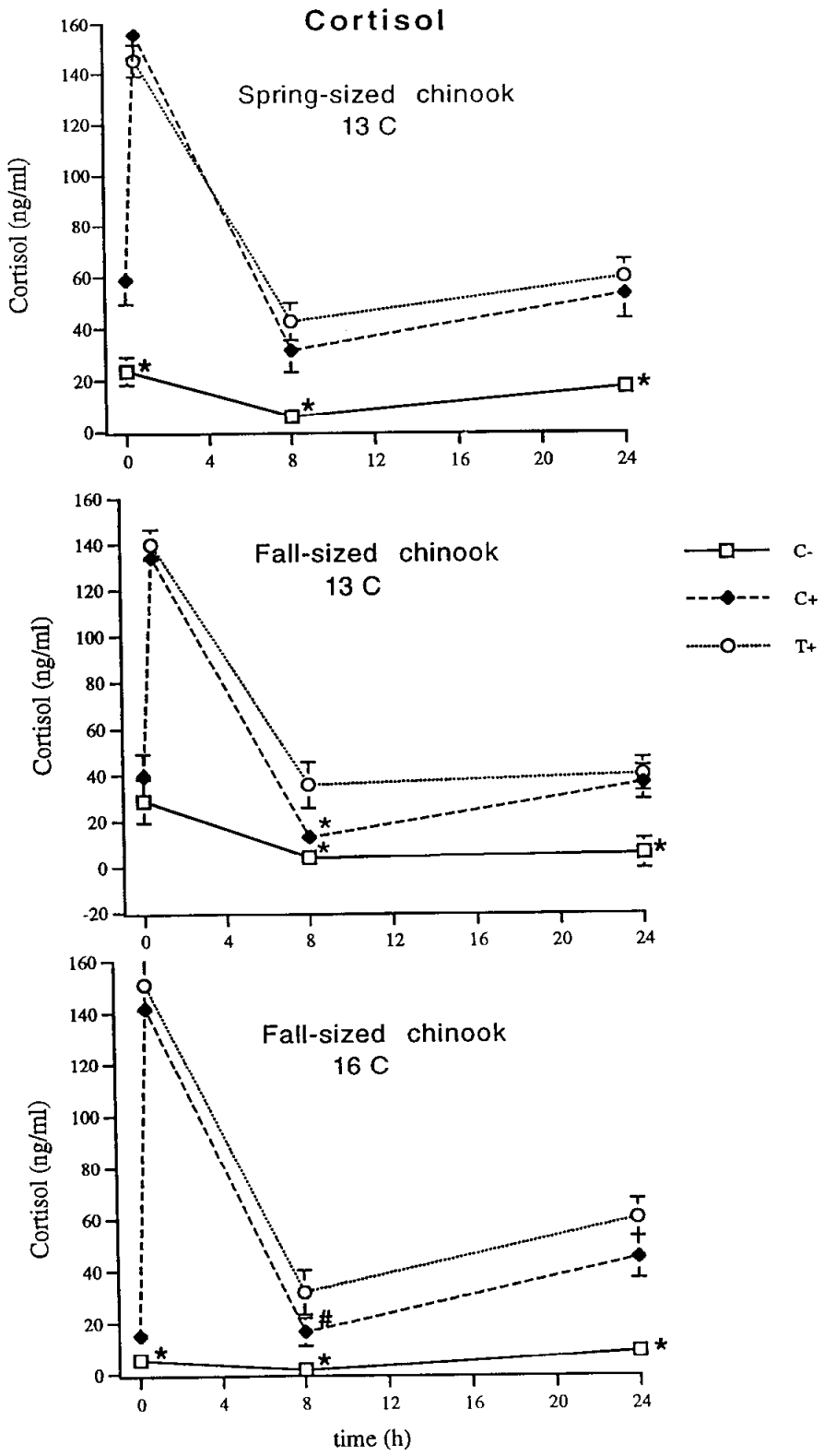

Figure 4. The cortisol levels (mean±SE) for spring-sized chinook smolts (13°C) and fall-sized chinook smolts (13 and 16°C) are shown at the timepoints 0, 5, 8, and 24 h. The negative control fish, C-, received no anesthesia and minimal handling. The other fish were anesthestised and either sham-tagged (C+) or balloon-tagged (T+). At 0.5 h, the balloon tags were removed from the T+ fish and the C+ fish were handled in a manner similar to that of the T+ fish. An * value is significantly different ($P \leq 0.05$) from the unmarked values at the same timepoint. A # indicates a value that is insignificantly different between the * and the unmarked value. N=12-18 values for C+ and T+ fish and 10-18 values for the C- fish at every timepoint.

Table 4. Mean values for plasma cortisol at 0, 0.5, 8, and 24 h for the unhandled negative control (C-), the sham-tagged (C+), and the balloon-tagged (T+) chinook salmon. The T+ fish were tagged at time 0 and the tags removed at 0.5 h. The C+ fish were handled in a manner similar to that of the T+ fish. An * indicates values significantly different ($P \leq 0.05$) from each other when comparisons between fish groups were made.

Fish	Temp (°C)	Time	Mean ±SE (ng/mL) C-	C+	T+
Spring	13	0	23.69 ±5.45	58.62 ±9.09	ND[1]
		0.5	ND	155.48 ±8.99	145.14 ±6.18
		8	5.66 ±1.82	31.39 ±8.36	42.38 ±7.2
		24	17.1 ±3.12*	52.95 ±9.73	59.52 ±6.63
Fall	13	0	28.75 ±9.41	39.4 ±9.65	ND
		0.5	ND	133.91 ±3.67	139.72 ±6.54
		8	3.99 ±1.78	12.82 ±3.47	35.49 ±10.01
		24	5.48 ±6.43*	36.09 ±7.3	39.74 ±7.23
Fall	16	0	5.5 ±1.07	15.01 ±2.88	ND
		0.5	ND	141.66 ±3.96	150.91 ±9.61
		8	1.49 ±0.24	16.34 ±5.53	31.69 ±8.61
		24	8.56 ±1.02	44.87 ±8.02	60.22 ±7.34

[1] ND = Not done.

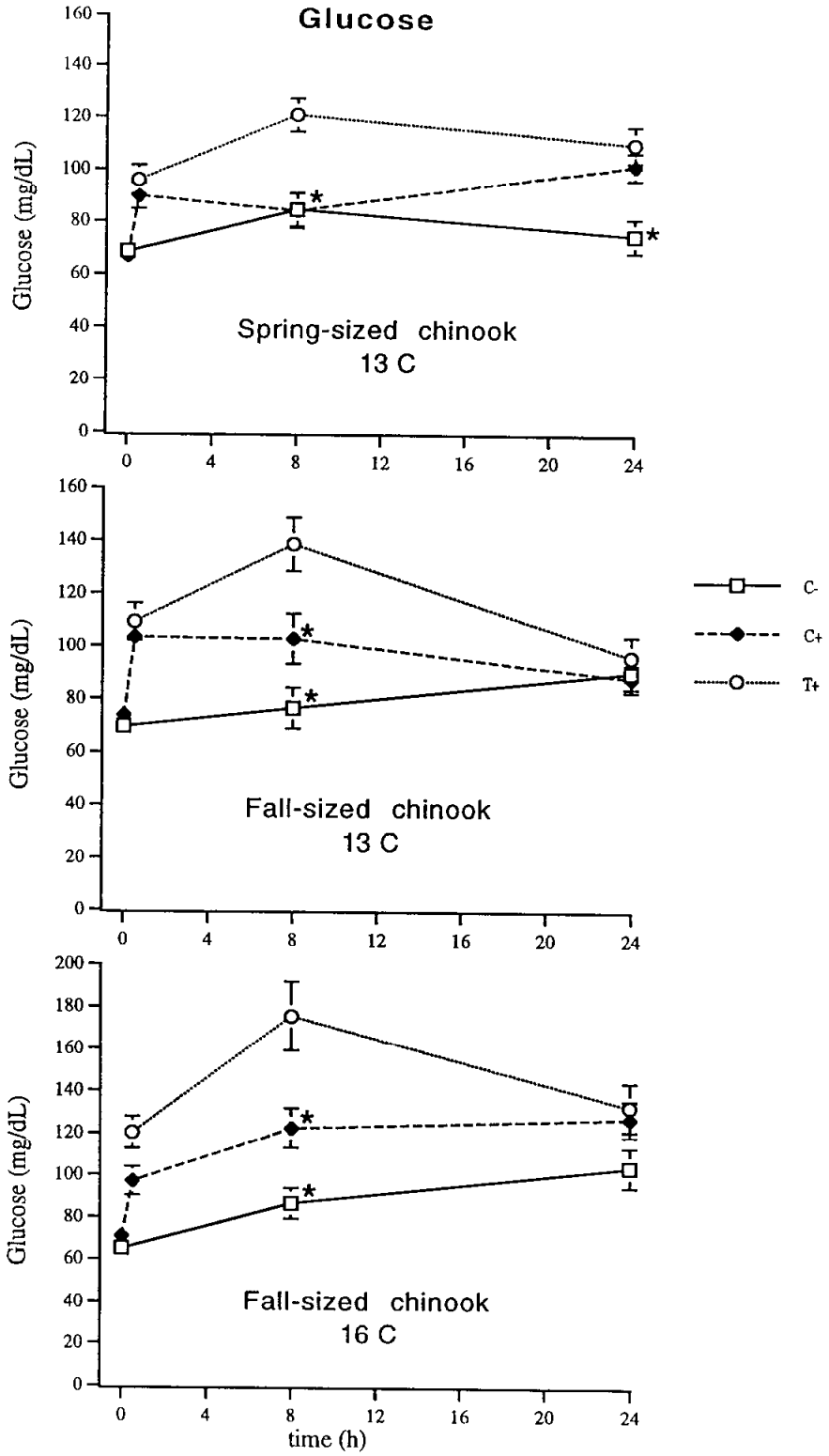

Figure 5. The glucose levels (mean±SE) for spring-sized chinook smolts (13°C) and fall-sized chinook smolts (13 and 16°C) are shown at the timepoints 0, 5, 8, and 24 h. The negative control fish, C-, received no anesthesia and minimal handling. The other fish were anesthestised and either sham-tagged (C+) or balloon-tagged (T+). At 0.5 h, the balloon tags were removed from the T+ fish and the C+ fish were handled in a manner similar to that of the T+ fish. An * value is significantly different ($P \leq 0.05$) from the unmarked values at the same timepoint. N=9-18 values for C+ and T+ fish and 5-18 values for the C- fish at every timepoint.

Table 5. Mean values for plasma glucose at 0, 0.5, 8, and 24 h for the unhandled negative control (C-), the sham-tagged (C+), and the balloon-tagged (T+) chinook salmon. The T+ fish were tagged at time 0 and the tags removed at 0.5 h. The C+ fish were handled in a manner similar to that of the T+ fish. An * or # indicates significantly different ($P \leq 0.05$) values (within a treatment) from each other when comparisons between fish groups were made.

Fish	Temp (°C)	Time	Glucose (mg/dL) mean ±SE C-	C+	T+
Spring	13	0	68.7 ±2.4	66.8 ±4.3	ND[1]
		0.5	ND	89.7 ±5.0	96.0 ±5.7
		8	84.7 ±6.3	84.3 ±6.6	120.9 ±6.3
		24	75.1 ±6.4	102.0 ±5.3	110.3 ±6.9
Fall	13	0	69.6 ±1.9	73.8 ±3.5	ND
		0.5	ND	103.4 ±4.6	109.2 ±7.0
		8	76.6 ±7.7	102.8 ±9.5	138.7 ±10.2
		24	90.2 ±6.1	87.9 ±5.2*	96.2 ±7.7#
Fall	16	0	65.0 ±2.4	70.9 ±4.2	ND
		0.5	ND	97.1 ±6.7	120.3 ±7.6
		8	86.7 ±7.4	122.5 ±9.4	175.6 ±16.4
		24	103.9 ±9.5	127.2 ±8.5*	132.7 ±11.4#

[1] ND=Not done.

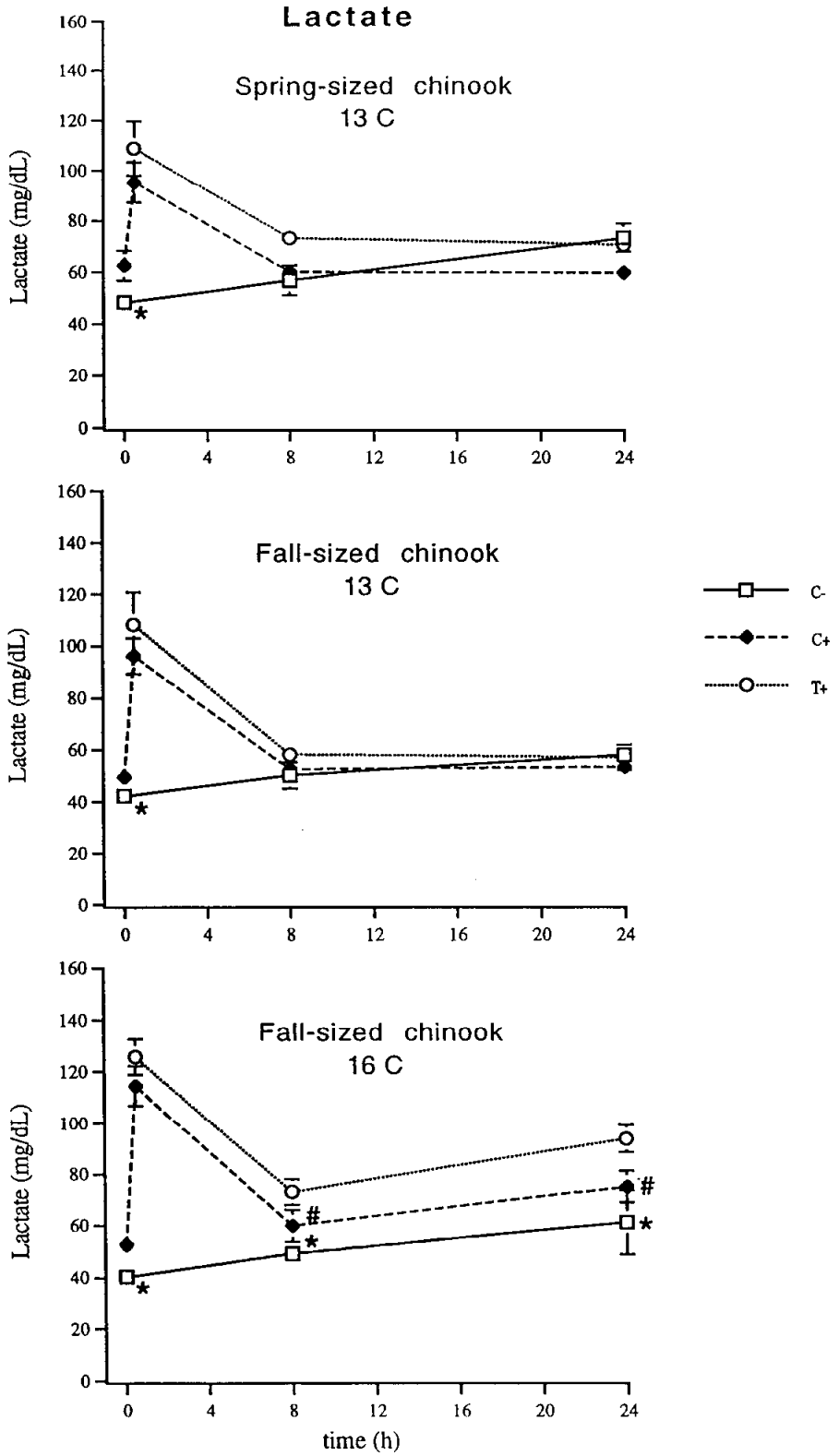

Figure 6. The lactate levels (mean±SE) for spring-sized chinook smolts (13°C) and fall-sized chinook smolts (13 and 16°C) are shown at the timepoints 0, 5, 8, and 24 h. The negative control fish, C-, received no anesthesia and minimal handling. The other fish were anesthestised and either sham-tagged (C+) or balloon-tagged (T+). At 0.5 h, the balloon tags were removed from the T+ fish and the C+ fish were handled in a manner similar to that of the T+ fish. An * value is significantly different ($P \leq 0.05$) from the unmarked values at the same timepoint. A # indicates a value that is insignificantly different between the * and the unmarked value. N=9-18 values for C+ and T+ fish and 7-18 values for the C- fish at every timepoint.

Table 6. Mean values for plasma lactate at 0, 0.5, 8, and 24 h for the unhandled negative control (C-), the sham-tagged (C+), and the balloon-tagged (T+) chinook salmon. The T+ fish were tagged at time 0 and the tags removed at 0.5 h. The C+ fish were handled in a manner similar to that of the T+ fish. An * indicates significantly different ($P \leq 0.05$) values from each other when comparisons between fish groups were made.

Fish	Temp (°C)	Time	Mean ±SE (ng/mL) C-	C+	T+
Spring	13	0	48.4 ±1.6	62.6 ±5.8	ND[1]
		0.5	ND	95.3 ±8.0	108.8 ±10.7
		8	57.0 ±5.7	60.2 ±4.8	73.5 ±4.0
		24	73.6 ±5.5	60.0 ±3.0	71.0 ±4.3
Fall	13	0	42.2 ±2.9	49.5 ±3.5	ND
		0.5	ND	96.1 ±6.9	108.3 ±12.6
		8	50.1 ±5.1	52.5 ±3.5	58.2 ±3.8
		24	58.0 ±4.1	53.3 ±3.9	57.1 ±4.9*
Fall	16	0	40.4 ±1.7	52.6 ±2.7	ND
		0.5	ND	114.4 ±7.7	125.7 ±6.9
		8	49.1 ±4.3	59.9 ±6.2	73.0 ±5.0
		24	61.2 ±12.3	75.1 ±6.1	93.9 ±5.4*

[1] ND=Not done.